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ScienceDirect
Carbohydrate Polymers 69 (2007) 562–578
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Method for determining the amylose content, molecular weights, and weight- and molar-based distributions of degree of polymerization of amylose and fine-structure of amylopectin ☆

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Received 9 December 2005; received in revised form 12 December 2006; accepted 15 January 2007
Available online 31 January 2007

Abstract

Cooked rice texture and other aspects of rice starch functionality are influenced by amylose and amylopectin content and structure. A method is described that uses high performance size exclusion chromatography coupled with multiangle laser light scattering and refractive index detectors to determine amylose content, molecular mass of amylose and the weight- and molar-based distributions of degree of polymerization (DP) of amylose and the fine structure of amylopectin. The method is relatively rapid with the coefficient of variation less than 5% for most aspects of the molecular characterization. It should find utility in research programs studying the association between starch molecular characteristics and functionality, and in starch-related genetics studies of diverse botanical sources.

Keywords: Amylose; Amylopectin; Chain length distribution; Degree of polymerization; Molecular weight; Starch; MALLS; SEC

1. Introduction

Rice starch composes approximately 90% of milled rice. It is made up of two major glucose polymers, amylose, which is a slightly branched, primarily long chain α(1-4) glucan, and amylopectin, which is highly branched through α(1 → 6) linkages. The amylose content of rice starch ranges from 0 to 30% (w/w). Cooked rice texture and rice starch functional properties are reported to be primarily impacted by amylose content (Bhattacharya, Sowbhagya, & Indudhara Swamy, 1982). However, evidence is building that variation in other aspects of rice kernels are also important determinants of rice cooking and processing quality (Bergman, Bhattacharya, & Ohtsubo, 2004). Some of these determinants include: water-soluble versus insoluble amylose content and debranched amylopectin chain length (CL) distribution (Qi, Texter, Snape, & Ansell, 2003; Tsai & Lii, 2000; Umemoto, Yano, Satoh, Shomura, & Nakamura, 2002; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003a; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003b). Examples of other starch characteristics that might influence rice functional properties, but have received limited attention, are molecular mass (M) and M distributions and branching parameters such as root mean-square radius and connectivity between branches. Various methods have been reported to determine these aspects of rice that are thought to control cooked rice texture and rice starch functionality, some of which are discussed below.

Amylose content has historically been determined by the iodine-binding procedure through either amperometric (Larson, Gilles, & Jennes, 1953), potentiometric (Banks & Greenwood, 1975), or spectrophotometric detection (Juliano, 1971). These methods are based on the ability of iodine to form a helical inclusion complex with amylose. However, iodine also binds with the amylopectin

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0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.01.018

(DP > 60), which causes overestimation of the amylose content. Furthermore, the phospholipids and free fatty acids compete with iodine in forming complexes with amylose, which tends to cause underestimation of the amylose content. Consequently, amylose content measured using this method has been termed amylose equivalents or apparent amylose. Methods to decrease the impact of the factors mentioned above on the iodine-binding procedure have been reported (Juliano et al., 1981; Perez & Juliano, 1978). A differential scanning calorimetric (DSC) method was reported that is based on the complex formation between amylose and added excess phospholipid and its resulting change in enthalpy during cooling (Mestres, Matencio, Pons, Yajid, & Fliedel, 1996). The sample defatting step prior to analysis reportedly is not necessary, however, overestimation of the amylose content is thought to result because the minimum CL for complex formation is below 30, thus some amylopectin might also be complexed (Gérard, Barron, Colonna, & Planchot, 2001). Gibson, Solah, and McCleary (1997) developed a method for amylose content that uses a lectin that interacts specifically with α-D-glucosyl units at multiple non-reducing end-groups and so forms a precipitate with amylopectin. The amount of carbohydrate in the soluble fraction is thus considered to be amylose. The amylose content of starch determined using the lectin-binding (concanavalin A) method is comparable to the amylose content in the water-soluble portion of the starch, but less than that in total starch, using the iodine method (Noosuk, Hill, Pradipasena, & Mitchell, 2003). Size-exclusion chromatography (SEC) has also been used to determine amylose content by quantitation of the amount of amylose relative to amylopectin. Comparable results in measured amylose content have been reported when SEC, iodine-binding, DSC and lectin-binding methods were compared (Batey & Curtin, 1996; Gérard et al., 2001).

The number-average M (M_n) of amylose has been determined using low-pressure SEC by performing off-line chemical analyses of collected fractions (Ramesh, Ali, & Bhattacharya, 1999a). This method is very time consuming, therefore its use discourages the study of large sample numbers. HPSEC-RI has been used to estimate M of amylose against a calibrated standard curve. This technique coupled with either low- or multi-angle laser light scattering (LALLS or MALLS) and RI detectors was used to determine the absolute M of both amylose and amylopectin polymers from different botanical sources (Bello-Pérez, Roger, Baud, & Colonna, 1998; Hizukuri, 1986; Hizukuri & Takagi, 1984; Ong, Jumel, Tokarczuk, Blanshard, & Harding, 1994). Using this system, You and Izydorczy (2002) resolved debranched amylose into a bimodal distribution, and characterized the molecular structures of the sub-fractions. Rice starch has not been evaluated using this methodology. Hanashiro and Takeda (1998) introduced a fluorophore to the reducing terminal of amylose, and then examined the molar-based distribution of amylose by fluorescence detection coupled to HPSEC-RI.

The weight-average M (M_w) of five fractions of amylopectin from different botanical sources were quantified by Hizukuri (1986) using an HPSEC-LALLS-RI system. These fractions were reported to represent exterior A and B1 chains within one cluster, and B2, B3, and B4 chains spanning 2, 3 and >4 clusters, respectively. Resolving amylopectin isoamylolysate into individual molecules by its CL or DP has been performed using several techniques. High-performance anion-exchange chromatography with pulsed-amperometric detection (HPAEC-PAD) has been used, but the detector response was not quantitative to the CL of the oligosaccharides (Koizumi & Fukuda, 1991). The addition of a post-column amyloglucosidase reactor to the HPAEC-PAD system increased the ability to quantitate oligosaccharides up to CL 77 (Wong & Jane, 1997). Grimm, Bergman, and Grimm (2003) reported the use of matrix-assisted laser-desorption ionization-time of flight mass spectrometry to quantify debranched rice starch DP, however, CL of only up to 45 glucose units could be resolved. Capillary electrophoresis, first reported by O'Shea, Samuel, Konik, and Morell (1998), is also used in the study of amylopectin CL distributions. The need to normalize the detector response to different CL compromises the ability to compare data from this technique across studies. Chiou, Fellows, Gilbert, and Fitzgerald (2005) have reported a normalization-independent plotting method to present the M distribution of amylopectin determined using capillary electrophoresis.

The functional attributes of rice starch are thought to involve both amylose and amylopectin and their interaction, thus analyses of the contents and molecular distributions of both polymers need to be performed. In addition, studies of structure and functionality require a large sample size representative of the world's rice germplasm, so the ease and reproducibility of determining all aspects of molecular structure needs to be taken into account. The purpose of this study was to develop and evaluate a relatively rapid method for determining the absolute amylose content, and the M_w and the weight- and molar-based distributions of average-DP of amylose and amylopectin. The utility of the method is demonstrated by the analyses of rice cultivars with wide ranges in apparent amylose content and gelatinization temperature.

2. Materials and methods

2.1. Rice

Eight rice cultivars with amylose content ranging from 0 to 25% were selected. These are 'Dixiebelle' and 'Kataktara' (high amylose-types, >24%), 'L201' and 'Dellmont' (intermediate amylose-types, 20–24%), 'Rico1' and 'Bengal' (low amylose-types, <20%), and 'Daw Dam' and 'Hsiao Wu Tsu Tsi' (glutinous-types, <5%). Among them Rico1, Bengal and Daw Dam are cultivars that gelatinize at low temperatures, and the rest at intermediate temperatures.

The cultivars were grown under field conditions in Beaumont, Texas, in the year 2001, using cultural management

practices common for the region. Samples were harvested at approximately 20% moisture, dried to 12% moisture, de-hulled, and milled. The milled rice was ground using a Cyclone Sample mill (UDY Corp., Boulder, CO) and sieved through 100-mesh.

2.2. Chemical analysis

All chemicals used were ACS reagent grade and purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise specified. Alkali spreading value (ASV) determination was based on the method of Little, Hilder, and Dawson (1958) using 1.7% (w/v) KOH and is used to predict gelatinization temperature (GT) of rice. The degree of spreading or disintegration of the grain was graded on a 7-point ASV scale of predefined characteristics which correspond to a certain range of GT (Juliano, 1985). The % apparent amylose content was determined using the iodine spectrophotometric method of Perez and Juliano (1978) and modified based on Webb (1972) for use with a continuous-flow analyzer (AutoAnalyzer 3, Seal Analytical, Mequon, WI). The analyses were performed with two replicates unless noted differently. Starch content of the solutions collected at four different steps of debranched starch preparation of Dixiebelle ($n = 6$) was determined using the total starch assay kit (AA/AMG 11/01) from Megazyme International Ireland Ltd. Co. (Wicklow, Ireland) (McCleary, Gibson, & Mugford, 1997). The % recovery was calculated by dividing total starch in solution by initial weight of starch (on dry wt basis) * 100. The starch moisture was determined by dividing the difference of (the fresh starch wt − the dry starch wt) by the fresh starch wt. The starch was dried by heating at 130 °C for 1 h.

2.3. Starch isolation

The starch was prepared using the alkaline deproteination method of Patindol, Wang, Siebenmorgen, and Jane (2003) with some modification. Rice flour of 1.5 g was suspended in 6 mL of 0.05 N NaOH at 4 °C for 1 h with shaking, spun at 250 × g (Model CR412, Jouan Co., Saint-Herblain, France) for 5 min, and the supernatant and the top sticky protein layer poured off. After three more times of deproteination, the flour was agitated overnight in 6 mL of 0.05 N NaOH at 4 °C. The starch was suspended in deionized water and neutralized with HCl, and washed three more times with deionized water, rinsed with methanol, and lyophilized. Starch lipid was removed with 85% (v/v) methanol using a Soxhlet apparatus for 16 h. The average protein content of the starch samples after deproteination was 1.82% (w/w FW) (FP-528, Leco Corp., St. Joseph, MI).

2.4. Debranching starch

A 22.5-mg (FW) sample of starch was gelatinized in 0.3 mL of dimethylsulfoxide (HPLC grade, Sigma–Aldrich, St. Louis, MO) at 80 °C for 10 min followed by boiling for 10 min. Next 1.2 mL of 0.625 M NaOAc, pH 4.0, was added into the starch suspension and the starch was debranched overnight at 40 °C with the addition of 15 μL of isoamylase (250 U/mL, Megazyme, Wicklow, Ireland). The next day, the debranched starch was boiled for 10 min to stop the enzyme activity, spun at 12,000*g*, and the supernatant of the starch hydrolysate was desalted with an ion-exchange resin (AG501-XB, Bio-Rad, Hercules, CA) at 45 °C with gentle shaking, for 30 min. Then to an aliquot of 900 μL of the starch hydrolysate, 100 μL of 10× mobile phase was added equilibrating the starch hydrolysate to 1× mobile phase (50 mM $NaNO_3$ and 0.02% NaN_3). This solution was then spun at 12,000*g* for 10 min, diluted to 1/10 with 1× mobile phase, and filtered through a 0.45 μm PVDF membrane (Waters, Milford, MA). The final starch concentration is 0.135% (w/v). The filtrate was kept in a 45 °C incubator with constant shaking until just before the injection (150 μL) to the size-exclusion columns. The analyses were performed with six replicates for Dixiebelle and L201, five for Kataktara, four for Dellmont, Bengal, Daw Dam, and Hsiao Wu Tsu Tsi, and three for Rico1.

2.5. Preparation and analysis of debranched amylopectin

The debranched amylopectin was prepared according to the method of Hizukuri (1985) with some modification. To an aliquot of 600 μL of the debranched starch (prepared from above), 54 μL of 1-butanol (HPLC grade, Sigma–Aldrich, St. Louis, MO) and 6 μL of 10× mobile phase were added, and then mixed, incubated at 30 °C for 2 h, and spun at 2000*g* for 5 min. A 250-μL aliquot of the supernatant was lyophilized and then solubilized in 25 μL of dimethylsulfoxide at 80 °C. Next 225 μL of distilled water and 250 μL of 1× mobile phase were added. The sample solution (0.675% w/v) was spun (15,000*g*), filtered (0.2 μm Anotop filter, Whatman, Maidstone, England), and injected (150 μL) onto the size-exclusion columns. The analysis was performed with four replicates for each cultivar.

2.6. Chromatography and detection systems

One guard column, and two analytical size-exclusion columns, KS-804 (8 × 300 mm, pore size 200 Å, particle size 7 μm) and KS-803 (8 × 300 mm, pore size 100 Å, particle size 6 μm) (Shodex Co., Japan) were connected in tandem. The debranched samples were fractionated through these size-exclusion columns (maintained at 70 °C) at 0.7 mL/min with the mobile phase of 50 mM $NaNO_3$ and 0.02% NaN_3 by the HPLC system (Waters Corp., Milford, MA), and detected by multi-angle laser light scattering (MALLS) (DAWN EOS, Wyatt Technology Corp, Santa Barbara, CA) and differential refractive index (RI) (Waters Corp., Milford, MA) detectors. The performance of the SEC columns was monitored by mea-

suring the number of theoretical plates using fructose. The RI calibration constant was measured with a series of NaCl standards. The 90°-photodiode detector of MALLS was calibrated using toluene (HPLC grade). The rest of the 17 photodiode detectors at all scattering angles were normalized relative to the 90° detector using dextran (Dextran Standard 25,000, Fluka). The volume delay between MALLS and RI was determined using bovine serum albumin. The M of the measured dextran standards 25,000 and 5000 using our system were 25,720 and 5812, respectively. The M of dextran 25,000 and 5000 was measured as 22,700 and 5700 by the manufacturer using low-angle laser light scattering detection, and 23,800 and 5220 using gel-permeation chromatography.

2.7. Data analysis

The ASTRA software was set to collect data at a 1-s interval, and each data point or slice is equivalent to 11.7 μL eluting volume through the column at a flow rate of 0.7 mL/min. The solute concentration of each slice is calculated by the ASTRA using the RI calibration constant, the RI voltage, and the differential refractive index increment (dn/dc):

$$c = (V \times \mathrm{RI_{cc}})/(dn/dc) \quad (1)$$

where c is the mass concentration of the solute, V is the RI voltage, and $\mathrm{RI_{cc}}$ is the RI calibration constant. The dn/dc value of 0.146 (mL/g) was used for the isoamylolysates of amylose and amylopectin (Motawia et al., 2005; Roger & Colonna, 1993). The determinations of M and the mean square radius of the solute at each slice by ASTRA software are based on the Zimm Equation (Zimm, 1948). For a chromatographic mode of MALLS detection when the solute concentration is low ($A_2 \to 0$), the Zimm Equation is put into several mathematical formalisms that ASTRA uses to extrapolate the expected linear function to zero angle, and the molecular mass (M) and the mean square radius (r^2) are obtained from the y-intercept and the slope, respectively. The three extrapolation methods are: (1) K^*c/R_θ (Zimm, 1948); (2) R_θ/K^*c (Debye, 1947); and $\sqrt{K^*c/R_\theta}$(Berry, 1966) vs. $\sin^2(\theta/2)$, where R_θ is the excess Rayleigh ratio, a ratio of the scattered and incident light intensities where the scatted light is in excess of that of the solvent, i.e., the scattered light of the solute at angle θ; c is the mass concentration of the solute; K^* is the optical constant $= 4\pi^2 n_0^2 (dn/dc)^2 \lambda_0^{-4} N_A^{-1}$, where n_0 is the refractive index of the solvent at the incident radiation (vacuum) wavelength (λ_0), dn/dc is the change in refractive index with the solute concentration at λ_0, and N_A is Avogadro's number.

The average M and the z-average mean square radius are calculated by ASTRA by summation of all the slices over one peak or specified eluted-fraction:

Number-average molar mass (M_n): $M_n = \sum c_i / \sum (c_i/M_i)$

Weight-average molar mass (M_w): $M_w = \sum (c_i M_i) / \sum c_i$

z-average mean square radius (Rg^2): $Rg^2 = \sum (c_i M_i \langle r^2 \rangle_i) / \sum (c_i M_i)$

where c_i, M_i, $\langle r^2 \rangle_i$ in the above equations are the mass concentration, molar mass, and mean square radius of the ith slice. The polydispersity value ($=M_w/M_n$) was also calculated.

The weight-average DP (DP_w) and number-average DP (DP_n) were calculated by dividing M_w and M_n with the anhydrous glucose M (162 g/mol), respectively. The weight-based distribution of the DP_n of amylose was obtained by dividing the amylose fraction into 16 DP_n subfractions along the elution volume of the HPSEC, and expressed weight per subfraction on a per gram starch basis. The molar-based distribution of the DP_n of amylose was obtained by dividing the calculated mass of each DP_n subfraction by the M_n of that subfraction. The % absolute amylose value was calculated by dividing the calculated mass of the amylose by the total calculated mass (amylose + amylopectin fractions).

The peaks of the large- and small-M fractions of debranched amylopectin (AmpF1 and AmpF2, respectively) were divided at the inflection point, and the M_w of AmpF1 and AmpF2, and the mass ratio of AmpF2 to AmpF1 determined. The fine structure of molar-based distribution of weight-average CL (CL_w) of amylopectin was characterized as moles ($=$mass$/M_w$) per CL_w subfraction on a per-gram amylopectin basis, where the 24 subfractions were divided from the debranched amylopectin in every 0.14 mL.

2.8. Statistical analyses

Variability in the data collection was determined by calculating the standard deviation and coefficient of variation of replicate analytical runs.

3. Results and discussion

3.1. MALLS and size exclusion chromatography

The amylose isoamylolysates have high polydispersity with their M_w spanning almost two orders of magnitude, and the Rg in the range of 10–100 nm, with the minimum measurable Rg of 10 nm for the detectors. We calculated the M_w and Rg of the amylose subfractions using three extrapolation methods, Zimm, Debye and Berry at the first and second order of polynomial fits. The results for Dellmont presented in Table 1 demonstrates that comparable M_w were obtained using the three extrapolation methods at either the first or second order of polynomial fits. Similar results were obtained for other high- and low-amylose types of rice amylose (data not shown). Variation in Rg values was observed among the three extrapolation methods, and the first order of polynomial fits gave a lower error rate than the second order (Table 1). Yokoyama, Renner-Nantz, & Shoemaker (1998) reported that all three

extrapolation methods obtained similar M_w and Rg results for a potato amylose sample (2×10^5 Da). However, the Zimm extrapolation method has lower precision of the polynomial fit for molecular sizes >100 nm (Shortt, 1993), and it has been suggested that it be used for molecules <50 nm (ASTRA, 2002). Therefore, we used the Berry extrapolation method with the first order polynomial fit for M_w and Rg determinations of amylose and its subfractions, whereas, the Zimm method with first order polynomial fit was used for characterizing debranched amylopectin.

The HPSEC-RI separates molecules by their hydrodynamic volumes and determines the M of polymers based on calibration of the column with standards of known M. However, the typical standards used for starch determination are pullulans, which are α-D-glucans possessing two-thirds (1 → 4) linkages and one-third (1 → 6) linkages. Neither the linear or branched amylose, nor the highly branched native amylopectin or debranched amylopectin have the same conformation as the pullulan standards, thus calibration of the SEC column with pullulans cannot accurately determine the M of either the native or debranched amylose or amylopectin. Therefore, we used MALLS coupled with an RI detector, which allows for the determination of the absolute M of molecules after separation through the SEC without column calibration.

Another problem reported to occur when analyzing starch with HPSEC is that starch molecules interact with the column polymers causing non-size separation of the molecules. For example, Chuang (1995) and Meehan (1995) reported that starch molecules eluted in an earlier volume than their actual hydrodynamic volume and poor reproducibility resulted. These problems have occurred in both organic-phase and aqueous-phase SEC and reportedly can be eliminated with the addition of an ionic modifier to the mobile phase (Meehan, 1995). Yokoyama et al. (1998) reported obtaining a linear curve of M vs. elution volume of dextran standards after adding LiBr to a dimethylsulfoxide solvent, separating with gel permeation chromatography and monitoring with MALLS and RI detectors. However, this solvent is not optimal for cereal starch because its use has resulted in an upward curvature of M plotted against elution volume, at high-elution volumes, for waxy corn, waxy rice and tapioca starch (Yokoyama et al., 1998). This finding stresses the importance of optimizing the solvent along with the polymers being analyzed using SEC or gel-permeation chromatography. This type of optimization procedure can be monitored using MALLS.

Another difficulty experienced when characterizing starch involves the associations between amylose molecules, which results in the formation of large aggregates that either fall out of solution prior to SEC separation, or are detected as high M super-molecules (Aberle, Burchard, Vorwerg, & Radosta, 1994; Bello-Pérez et al., 1998; Roger & Colonna, 1993). For example, a drastically changed chromatogram as well as decreases in the M_w and Rg of solubilized starch after 24 h were observed and suggested to be due to the removal of amylose aggregates by a filtration step prior to sample injection (Bello-Pérez et al., 1998). Roger & Colonna (1993) observed high light-scattering peaks with no corresponding RI response, at low elution volume, in the study of the M of leached corn amylose using HPSEC and MALLS. These amylose aggregations appear to be concentration dependent and their M differs based on the botanical source with the results reported to be from 3 to 35 times larger than expected (Aberle et al., 1994). The possible mechanisms leading to the formation of these aggregates include inter- and intra-molecular cross linking via double helix formation of amylose.

High M super-molecules were detected in our study of rice starch. Their formation was determined to be time dependant after we tested the effect of sample concentration and holding time prior to injection (Fig. 1a–d). These high-M polymers were not due to the un-debranched amylopectin since they were not detected in the sample run right after preparation. For example, Fig. 1a–d shows the same sample re-injected at 0 h (right after preparation), 3, 7 and 11 h after preparation. Increasing signals from the MALLS were detected for the polymers eluted between 13 and 14.4 mL as the storage time increased; while there were very small changes in the RI responses. The calculated M_w of the amylose fraction eluted between 13.6 and 15.6 mL was 1.22×10^6 g/mol at 0 h and increased to 2.58×10^6 at 7 h. By plotting the log Rg vs. log M_w

Table 1
Comparison of three extrapolation methods for the determinations of weight average molecular mass (M_w) and z-average root-mean-square radius (Rg) of Dixiebelle amylose sub-fractions

Order of polynomial fit	Extrapolation method	M_w ($\times 10^{-6}$ g/mol)		Rg (nm)
		amy1	amy2	amy1
First-order fit	Zimm	1.144 (0.4%)[a]	0.214 (0.7%)	54.4 (0.8%)
	Debye	1.102 (0.6%)	0.214 (0.7%)	40.0 (1.6%)
	Berry	1.132 (0.3%)	0.214 (0.7%)	49.6 (0.7%)
Second-order fit	Zimm	1.140 (0.5%)	0.219 (0.9%)	52.7 (3.0%)
	Debye	1.131 (0.5%)	0.218 (0.9%)	48.2 (2.4%)
	Berry	1.139 (0.5%)	0.219 (0.9%)	52.0 (2.6%)

[a] Precision of polynomial fit.

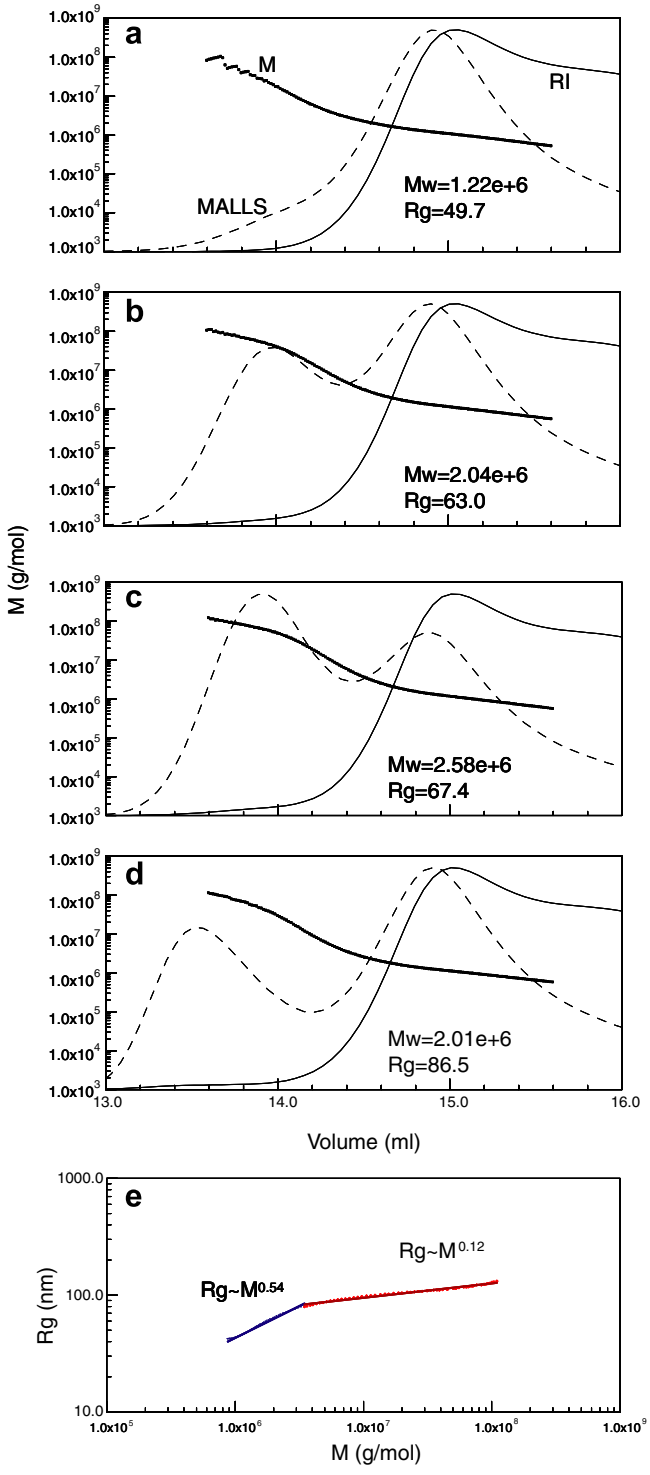


Fig. 1. Time course stability study of isoamylolysates of Dellmont starch. The profiles of molecular mass (M), MALLS and RI responses of the portions of the amylose fraction of isoamylolysates of Dellmont starch eluted through HPSEC at (a) 0, (b) 3, (c) 7 and (d) 11 h after preparation. The values of weight-average M (M_w) and root-mean square radius (Rg) are for the amylose fraction eluted between 13.6 and 15.6 ml. Panel (e) is a molecular conformation plot of the sample from 11 h-after-preparation.

(Fig. 1e), two types of polymer conformation were observed at a point of change at M_w of $\sim 3 \times 10^6$ (g/mol) ($\sim$14.4 mL in Fig. 1d). Prior to that point a random coil conformation was observed ($Rg \sim M^{0.54}$), whereas, after this a more compact structure was found ($Rg \sim M^{0.12}$). Aberle et al. (1994) suggested that aggregates with increasing M, but with little increase of Rg, are formed by the side-by-side alignment of amylose double helices.

In addition to evaluating the effects of starch concentration and holding time on super-molecule formation, we also studied the effects of the mobile phase (i.e. sodium nitrate or water) combined with the sulfonated polystyrene-divinyl benzene SEC columns, sample holding-temperature and agitation, and column temperature. Fig. 2a shows representative RI and MALLS' M_w profiles vs. the elution volume of HPSEC of the debranched rice starch from Dellmont (an intermediate amylose cultivar) using the optimized method reported in Section 2. The linear line of M against elution volume indicates that suitable mobile phase composition, sample holding time and column conditions for the elution of polymers through the HPSEC were achieved with our procedure. That is, no large aggregated super-molecules were detected from the MALLS, and the profiles of the HPSEC chromatogram detected by both detectors were unchanged for at least eight hours after the sample preparation (data not shown). The dependence of size (Rg) upon M ($Rg \sim M^{0.48}$) found for the debranched starch corresponds to a random coil conformation (Fig. 2b). Molecular conformation cannot be determined for polymers with $Rg < 10$ nm, because there is no angular variation of the scattered light intensity for an incident light of wavelength 690 nm. Since SEC resolves molecules by their hydrodynamic volumes, the semi-log plot of M_w vs. elution volume could be used to observe the conformations of the eluted molecules with $Rg < 10$ nm (Fig. 2a). The optimal set of conditions is as follows: starch concentration was 0.135% (w/v) for total starch and was 0.675% for debranched amylopectin, and the samples were kept at 40 °C with agitation prior to SEC; the columns' temperature was 70 °C; and the mobile phase was 50 mM $NaNO_3$ and 0.02% NaN_3.

3.2. Starch solubility

Rice starch solubility in DMSO and the % recovery during the debranching steps were determined using Dixiebelle and analyzed by a total starch method (McCleary et al., 1997). Samples were collected at step (1): after the overnight-isoamylase-digestion, step (2): after centrifuging at 12,000g, step (3): after desalting, and step (4): after filtration and right before injection onto the column. The recoveries at each step were: (1) 96.5 ± 3.2, (2) 96.7 ± 4.7, (3) 90.9 ± 4.0, and (4) 88.9 ± 4.6. The % recoveries of step (1) and (2) samples represent the results of starch solubility which are approximately 97% in our preparation. The % recoveries of collected samples dropped slightly at steps (3) and (4) through preparation. Using ASTRA we calculated the mass of the eluted samples after SEC with Eq. (1). The % recovery of the SEC-eluted isoamylolysates of Dixiebelle starch was $83.0 \pm 1.4\%$. The column recovery

Table 2
Amylose content determination by iodine-spectrophotometric and SEC methods and the total starch recovery of eight rice cultivars

Cultivar	Iodine % Apparent amylose	SEC % Absolute amylose	Amylose content of SEC to iodine %	% recovery after SEC
Dixiebelle				
Mean	24.90	23.79	95.5	83.1
CV	0.57	1.42		1.4
Kataktara				
Mean	25.45	24.06	94.5	82.5
CV	1.39	1.94		1.3
Dellmont				
Mean	22.75	20.70	91.0	83.6
CV	0.31	0.67		1.5
L201				
Mean	21.30	17.94	84.2	81.3
CV	1.94	2.38		2.8
Rico1				
Mean	18.60	16.34	87.8	78.1
CV	0.00	2.49		1.5
Bengal				
Mean	12.75	9.26	72.6	82.1
CV	0.55	2.94		2.8
Hsiao Wu Tsu Tsi				
Mean	0.10	0.00	NA[a]	83.7
CV				3.7
Daw Dam				
Mean	0.00	0.00	NA[a]	85.6
CV				0.4

[a] NA, not applicable.

ranged from 0% to 30%). However, the amylose content as determined by taking the percentage area of the amylose-derived peak needed to be multiplied by a factor of 1.58 (Batey & Curtin, 1996). Visible precipitation of their preparation of high-amylose amylomaize starch (>30%) was observed resulting in lower amylose content determination. Unfortunately, no recovery rate was determined. Gérard et al. (2001) compared four different amylose determination methods using native starch from different botanical sources. The amylose concentrations of native starch, determined using low-pressure SEC, ranged from 83% to 110% of those obtained using the Con A and iodine binding capacity (IBC) methods. The % yield after starch solubilization and filtration were >95%, while the chromatographic yields were >90%. Ramesh, Mitchell, Jumel, & Harding (1999b), however, reported that the amylose content obtained from the HPSEC of debranched rice starch were <50% of those obtained from iodine method and suggested that the amylose content in rice starch is much lower than often presented in the literature. Neither % recovery nor how the amylose content was determined in their HPSEC method was reported. Our results from the comparison of the two methods (HPSEC-MALLS-RI and iodine binding methods) for rice starch amylose content determination are comparable to those of Batey & Curtin (1996) and Gérard et al. (2001), and the % absolute amylose content of rice is within the range of 85–95% of the apparent amylose content. The starch solubilities and recoveries are within a similar range to that of Gérard et al. (2001). We also demonstrated that the % recoveries of rice starch, which is necessary for starch characterization studies, can be obtained using the HPSEC-RI method.

Because of the ease of sample preparation and relatively high throughput of the iodine spectrophotometric method, effort has gone into improving the original assay's lack of stability and toward minimizing the effects of factors known to influence amylose content determinations (Juliano, 1971; Juliano et al., 1981; Perez & Juliano, 1978; Williams, Wu, Tsai, & Bates, 1958). But, none of these modifications removed the error inherent in the method due to the assumption that all rice samples contain 90% starch, similar starch lipid content and similar interference from amylopectin. The operator- and laboratory-dependence of these iodine-binding methods have been reported (Batey & Curtin, 1996). Superior methods have been sought. Among them, SEC methods have been reported to be superior due to their absolute amylose determination, lack of interference from lipids, and supposed power to resolve amylose and amylopectin using their differences in hydrodynamic volume. Grant, Ostenson, & Rayas-Duarte (2002) used HPSEC to determine the absolute amylose content of native (un-debranched) wheat starch. Low-pressure SEC reportedly provides better resolution between

amylopectin and amylose than HPSEC when used to analyze native starch, but it is a very lengthy procedure (Gérard et al., 2001). Fully resolving these two polymers in their native forms might be hard to achieve since the ranges of DP of these two rice polymers overlap. Amylopectin has a wide M distribution reportedly ranging from 22,000 to 700 DP and the amylose distribution from 3100 to 230 DP (Hanashiro & Takeda, 1998; Takeda, Shibahara, & Hanashiro, 2003). In addition, SEC separates polymers based on their hydrodynamic volume. The highly branched native amylopectin polymer has a smaller hydrodynamic volume than that of amylose of the same M, thus, at the same elution volume amylopectin would have a higher DP than that of amylose. Also, some amylose has a branched structure that makes it a fraction between amylopectin and linear amylose. Thus, fully resolving the amylopectin and amylose in their native form using SEC would be a challenge. Takeda et al. (2003) demonstrated that the subfractions of non-debranched rice amylopectin with large, medium and small number-average DPs all gave similar unit chain distribution with DP < 100 after the treatment with isoamylase. This suggested that resolving these two starch polymers after an isoamylase digest should give better results than separating their native forms using HPSEC. Therefore, we used debranched starch for absolute amylose content determination.

3.4. Molecular characterization of amylose

Three different mass fractions of amylose were observed. Their distributions differed among the samples analyzed (Fig. 3). The molecular characterizations of the amylose and its subfractions, amy1 (elution volume 11.2–12.6 mL), amy2 (elution volume 12.6–14.0 mL), and amy3 (elution volume 14.0–15.4 mL) of six cultivars are presented in Table 3. The specific parameters determined included: weight percent, M_w (DP_w), M_n (DP_n), polydispersity, and Rg. Duplicates of the molecular parameters for the amylose fraction and its subfractions were reproducible (coefficients of variation <5%). A few exceptions were observed in the M values of subfraction amy3. This is likely due to the low quantity of, and smaller M of, the polyglucan in this fraction, which increased the variation in RI and MALLS responses. The range of M_w and M_n of amylose was 5.1–6.9 × 10^5 and 1.4–1.8 × 10^5, respectively, for six cultivars varying in amylose content. Our M_w and M_n values are approximately two to three-fold less than what Ong et al. (1994) reported for one waxy rice, and are comparable to the high end of the M_w range and to the low end of M_n range reported for four rice cultivars studied by Ramesh et al. (1999b). However, no subfractions of amylose were resolved or reported using their HPSEC systems. The native amylose of rice and maize h been reported to contain three major molecular speci observed by both the molar- and weight-based distr of a SEC-fluorescent detection system with re labeling of amylose (Hanashiro & Takeda, 19 of rice debranched amylose ranged from 84 3) which is slightly lower than other repo of native rice amylose (i.e. 920–1410 chemistry with the Park–Johnson detection with calibrated standa 1998; Hizukuri, Takeda, & M what has been reported, na with isoamylase slightl native amylose fracti resolved by SEC (T

The Fig. 4a distributions amylose c are exp reflec pa

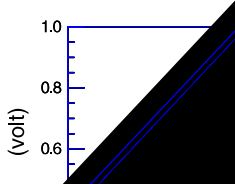


Table 3
Molecular characteristics of isoamylase-debranched amylose fraction and subtractions from six rice cultivars

Molecular characteristics[a]	Cultivar	Amylose			amy1			amy2			amy3		
		Mean	SD	CV (%)[b]	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
Weight % (g/g starch)	Dixiebelle	23.8	0.34	1.4	8.0	0.22	2.7	11.2	0.16	1.5	4.6	0.10	2.1
	Kataktara	24.1	0.47	1.9	6.7	0.26	3.8	12.4	0.17	1.4	4.9	0.07	1.4
	Dellmont	20.7	0.14	0.7	7.8	0.09	1.1	9.4	0.08	0.8	3.5	0.04	1.0
	L201	17.9	0.43	2.4	6.5	0.14	2.1	8.2	0.28	3.4	3.3	0.09	2.7
	Rico1	16.3	0.41	2.5	6.8	0.15	2.2	7.2	0.20	2.8	2.4	0.07	3.1
	Bengal	9.3	0.27	2.9	3.3	0.15	4.5	4.3	0.11	2.6	1.6	0.03	1.7
M_w ($\times 10^3$ g/mol)	Dixiebelle	508	1.37	2.7	1156	3.01	2.6	227	0.71	3.1	55.7	0.45	8.1
	Kataktara	416	0.89	2.1	1022	2.83	2.8	230	0.81	3.5	54.3	0.18	3.4
	Dellmont	591	0.94	1.6	1256	1.80	1.4	238	0.25	1.1	61.6	0.32	5.3
	L201	625	3.39	5.4	1397	6.78	4.9	227	0.65	2.9	60.6	0.72	12.0
	Rico1	692	1.62	2.3	1402	2.91	2.1	228	0.42	1.8	58.4	0.35	6.1
	Bengal	632	3.17	5.0	1435	8.25	5.8	228	0.39	1.7	64.6	0.89	13.8
DP_w	Dixiebelle	3134			7133			1398			344		
	Kataktara	2569			6306			1420			335		
	Dellmont	3646			7755			1466			380		
	L201	3859			8626			1398			374		
	Rico1	4273			8654			1409			360		
	Bengal	3900			8855			1405			399		
M_n ($\times 10^{-3}$ g/mol)	Dixiebelle	148	0.89	6.0	835	2.96	3.5	179	0.52	2.9	52.1	0.52	10.0
	Kataktara	137	0.39	2.9	803	2.23	2.8	181	0.64	3.5	50.4	0.17	3.4
	Dellmont	174	0.58	3.3	896	0.72	0.8	189	0.30	1.6	58.8	0.36	6.1
	L201	164	1.57	9.6	909	2.08	2.3	180	0.75	4.2	57.9	0.80	13.8
	Rico1	181	0.97	5.4	913	1.35	1.5	180	0.41	2.3	55.3	0.45	8.1
	Bengal	172	1.57	9.1	900	2.26	2.5	182	0.42	2.3	62.0	1.01	16.3
DP_n	Dixiebelle	913			5156			1107			321		
	Kataktara	847			4959			1116			311		
	Dellmont	1077			5532			1165			363		
	L201	1014			5612			1110			357		
	Rico1	1118			5635			1113			341		
	Bengal	1062			5558			1122			383		
Polydispersity (M_w/M_n)	Dixiebelle	3.4	0.24	7.0	1.4	0.03	2.2	1.3	0.01	0.9	1.1	0.02	2.1
	Kataktara	3.0	0.04	1.2	1.3	0.02	1.2	1.3	0.00	0.3	1.1	0.00	0.3
	Dellmont	3.4	0.14	4.1	1.4	0.01	0.8	1.3	0.01	0.6	1.0	0.01	0.9
	L201	3.8	0.20	5.2	1.5	0.04	2.7	1.3	0.02	1.3	1.0	0.02	1.8
	Rico1	3.8	0.12	3.1	1.5	0.01	0.8	1.3	0.01	0.5	1.1	0.03	2.7
	Bengal	3.7	0.46	12.4	1.6	0.05	3.4	1.3	0.02	1.6	1.0	0.02	2.4
Rg (nm)	Dixiebelle				39.2	0.75	1.9						
	Kataktara				36.8	0.53	1.4						
	Dellmont				41.5	0.40	1.0						
	L201				42.4	0.93	2.2						
	Rico1				43.2	0.87	2.0						
	Bengal				41.5	0.55	1.3						
nmoles/g starch	Dixiebelle	1600	130	8.1	110	5	4.3	642	13	2.1	849	118	13.9
	Kataktara	1799	76	4.2	106	6	6.0	703	28	4.1	991	42	4.3
	Dellmont	1247	36	2.9	98	1	0.7	520	6	1.1	629	31	4.9
	L201	1109	87	7.8	85	4	4.9	465	21	4.5	559	82	14.7
	Rico1	930	70	7.5	84	3	3.5	411	17	4.2	435	51	11.7
	Bengal	584	66	11.3	41	2	4.9	247	10	4.0	295	59	20.0

[a] M_w and M_n are weight and number average molecular weight, respectively; DP_w and DP_n are weight and number average degree of polymerization, respectively, and were converted by dividing the M_w and M_n by 162, the molecular weight of anhydrous glucose, respectively.
[b] CV (%): coefficient of variation; calculated by dividing SD/mean × 100.

one of each amylose type, relative to Dellmont, are presented in Fig. 5. From the molar distribution, the high amylose-type and the low amylose-type mainly differed quantitatively, relative to an intermediate amylose-type, in the smaller-DP_n range of the linear chains. L201, an intermediate amylose-type cultivar, which has less amylose content than Dellmont, has a lower molar proportion across the whole DP_n range relative to Dellmont.

The HPSEC-RI system has been used to study the weight-based DP distributions of high M of amylose and

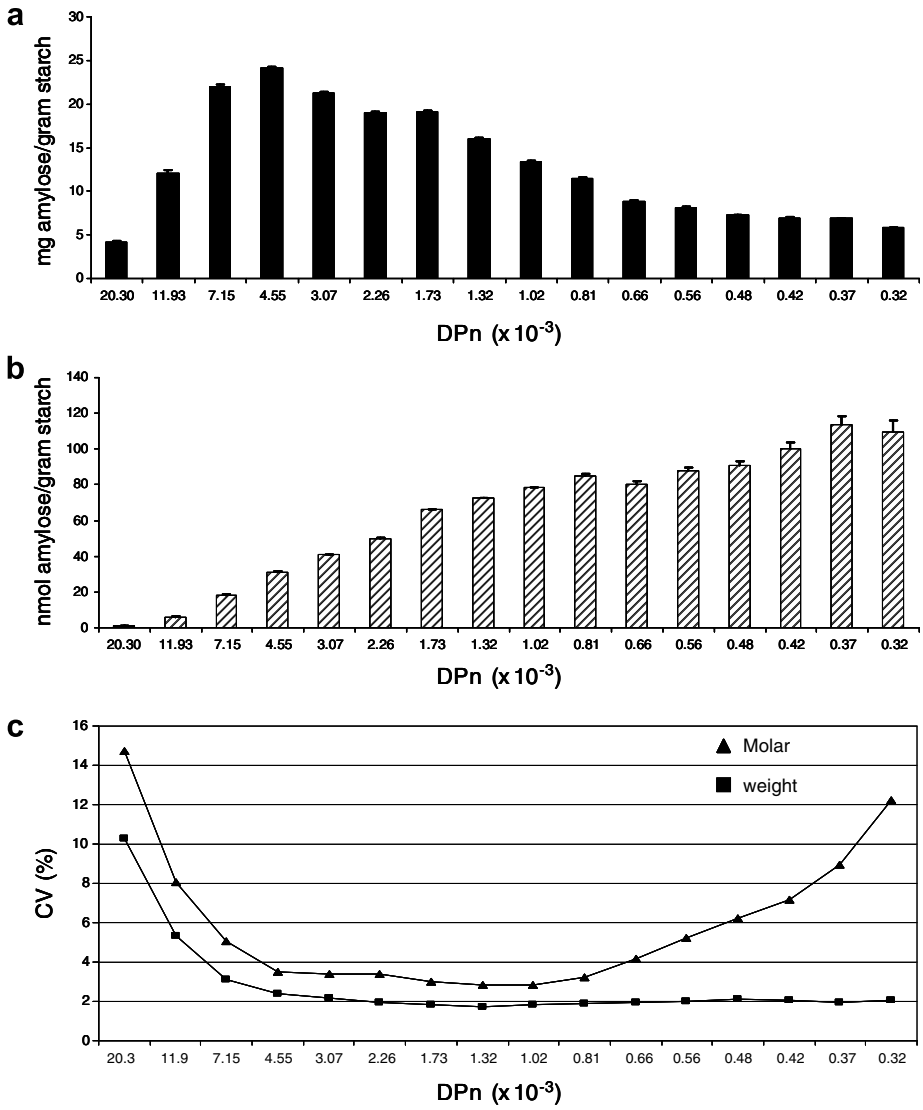


Fig. 4. Weight- (a) and molar-based (b) distributions of the number average degree of polymerization (DP_n) of isoamylase-debranched amylose of Dellmont, and the average of the coefficient of variations (CV, %) (c) of these distributions of six rice starches: Dixiebelle, Kataktara, Dellmont, L201, Rico1 and Bengal.

amylopectin. In comparison, high M polysaccharides can be quantitated either on a weight- or a molar-based distribution by introducing a fluorophore to the reducing terminal of amylose and evaluating its distribution with fluorescence detection coupled to HPSEC-RI (Hanashiro & Takeda, 1998). To use this technique, samples must be labeled and the construction of a standard curve of detection responses to the labeled standards created prior to fractionation. This procedure requires that the standards have the same conformation as the polymers analyzed. O′Shea et al. (1998) labeled pullulan standards of DP 70–1300 with a charged fluorophore and electrophoresed the labeled products on a polyacrylamide gel. This method would allow the study of the molar-based distribution of amylose if the labeling efficiency and solubility of amylose during labeling within this DP range could be established. In the present paper we demonstrated that the use of HPSEC-MALLS-RI system is capable of characterizing the M_w of amylose and its subfractions without standards, and capable of evaluating the weight- and molar-based DP distributions of amylose.

3.5. Molecular characterizations of amylopectin fine structures

The Fig. 6 presents the M and RI weight-based distributions vs. elution volume of the amylopectin isoamylolysates of Bengal and L201. Two subfractions, the high-(AmpF1) and the low-(AmpF2) hydrodynamic-volume fractions, with an inflection point at elution volume of 17.05 mL were observed. Good repeatability was obtained for the M_w determination of AmpF1 and AmpF2 (CV < 2%) and for the mass ratio of AmpF2/F1 (CV < 6%) (Table 4). The DPn of AmpF1 and AmpF2 and the mass ratios of AmpF2/F1 were within the range of those reported by others (Hizukuri, 1985, 1986; Ong et al., 1994). As reported by

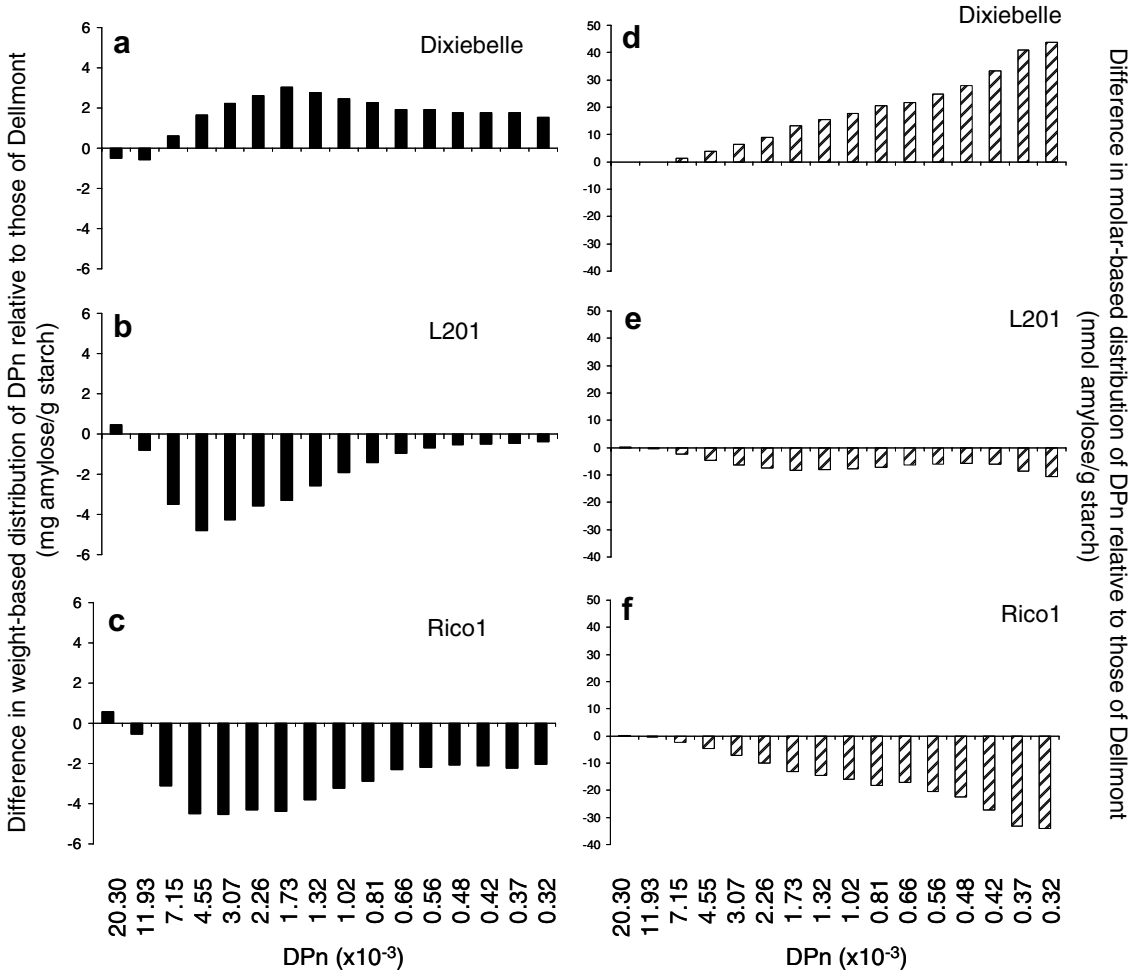


Fig. 5. Differences in the weight-s (a–c) and molar-based (d–f) distributions of the number average of degree of polymerization (DP_n) of amylose fractions of three isoamylase-debranched rice starches relative to those of Dellmont.

Hizukuri (1986), AmpF1 contained long B2, B3 and B4 chains that extended into 2, 3 and >3 clusters, respectively, and AmpF2 contained short exterior chains of A and B1 that make a single cluster. Differences in the RI response profiles were seen in AmpF2 between a low-GT cultivar, Bengal, and an intermediate-GT cultivar, L201 (Fig. 6). However, only a slight difference in the peak DP of AmpF2 between the two types of rice was observed, and the weight-average DP of AmpF2 did not differ among these two cultivars.

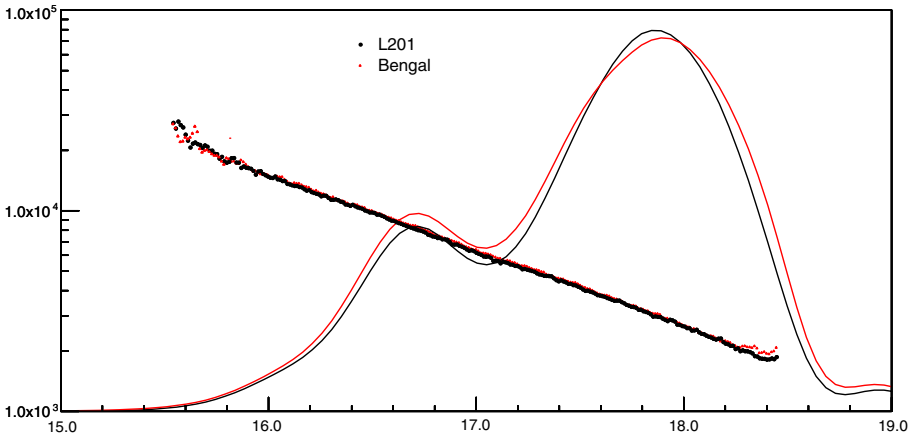


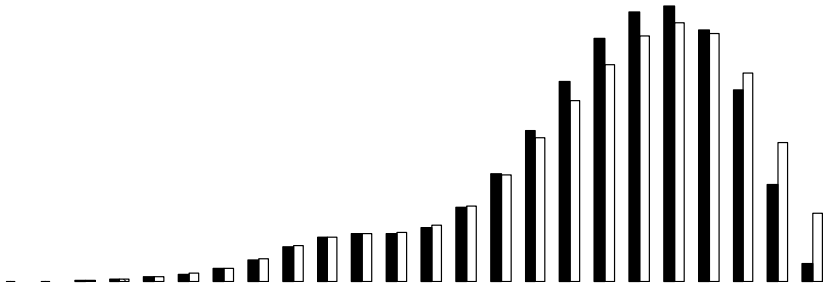
Table 4
Weight average molecular mass (M_w) and mass ratio of subfractions of isoamylase-debranched amylopectin

Cultivar	AmpF1[a]				AmpF2[a]				Mass ratio: AmpF2/F1
	M_w	(DP_w)[b]	M_w peak	(DPpeak)[b]	M_w	(DP_w)	M_w peak	(DPpeak)	
Dixiebelle									
Mean	9255	(57.1)	8147	(50.3)	3339	(20.6)	3018	(18.6)	2.86
SD	176		113		39		49		0.16
CV (%)	1.90		1.39		1.17		1.64		5.51
Kataktara									
Mean	9315	(57.5)	8178	(50.5)	3360	(20.7)	3013	(18.6)	2.96
SD	117		99		30		30		0.15
CV (%)	1.26		1.21		0.90		0.99		5.24
Dellmont									
Mean	9175	(56.6)	8137	(50.2)	3332	(20.6)	2984	(18.4)	3.16
SD	203		53		26		36		0.10
CV (%)	2.21		0.66		0.77		1.21		3.14
L201									
Mean	9251	(57.1)	8175	(50.5)	3363	(20.8)	3014	(18.6)	3.00
SD	88		48		34		49		0.15
CV (%)	0.95		0.58		1.00		1.63		4.94
Rico1									
Mean	9213	(56.9)	8112	(50.1)	3324	(20.5)	2909	(18.0)	2.99
SD	77		49		38		35		0.15
CV (%)	0.83		0.60		1.13		1.20		4.94
Bengal									
Mean	9295	(57.4)	8217	(50.7)	3348	(20.7)	2924	(18.0)	2.94
SD	145		76		30		12		0.15
CV (%)	1.56		0.92		0.89		0.40		5.26
Hsiao Wu Tsu Tsi									
Mean	9479	(58.5)	8303	(51.3)	3389	(20.9)	2990	(18.5)	3.11
SD	127		48		35		43		0.14
CV (%)	1.34		0.58		1.02		1.44		4.51
Daw Dam									
Mean	9315	(57.5)	8127	(50.2)	3351	(20.7)	2901	(17.9)	3.24
SD	128		80		18		22		0.10
CV (%)	1.37		0.98		0.55		0.75		2.98

[a] AmpF1 and F2 are the subfractions of higher and lower hydrodynamic volume of amylopectin isoamylolysate, respectively.
[b] DP_w and DP peak are the weight-average and peak degree of polymerization obtained by dividing M_w/162 and M_w peak/162, respectively. The value of 162 is the anhydrous glucose molecular weight (g/mol).

The molar-distributions of the amylopectin CL_w of L201 and Bengal are presented in Fig. 7a. Since HPSEC resolves molecules by their hydrodynamic volume, each slice contains heterogeneous molecules within a narrow range of *M*. L201 has a higher molar proportion between CL 15 to 25 and less between 9 to12 relative to Bengal. By comparing the molar distributions of CL_w of L201 and Bengal to that of a low GT cultivar, Rico1, the portion of the chains that were different between the L201 and Bengal in the A to B1 exterior short chains can be more easily seen (Fig. 7b). These results are comparable to those reported using HPAEC-PAD in that the higher GT cultivars are rich in chains of $12 < DP < 24$ and lower in chains of $DP < 11$ (Umemoto et al., 2002). The weight- and molar-chain distributions of amylopectin that we observed were up to $CL > 116$. The Table 5 presents total moles of each subfraction/*g* of amylopectin and their standard deviation and CV (%). The molarities of the CL_w distributional regions, which reflected the maximum molar differences of B1 (CL_w 25–15) and A chains (CL_w 12–11) between low- and intermediate GT cultivars (Fig. 7b), were summed and their ratio calculated. The intermediate GT cultivars, which have ASV values around 4–5, have higher ratios of B1/A than those of low GT cultivars, which have ASV values around 6–7. The waxy rice cultivars, which have non-detectable amylose content, have a lower ratio of B1/A than those of non-glutinous rice varieties of the same GT.

Methods are available for studying the molar-based distribution of short oligoglucans, but not without restraint. With the application of HPAEC, the debranched amylopectin was resolved into their individual molecules by their CL but, due to use of PAD detection, is not quantitative for oligoglucans with CL beyond 14 (Koizumi & Fukuda, 1991). The HPAEC-PAD with an addition of a post-column amyloglucosidase reactor system improves the quantitative determination up to CL 77 (Wong & Jane, 1997). These two methods measure essentially weight-based distributions. The attachment of the charged fluorophore as a label to the



reducing end of the debranched amylopectin also allows the separation of the fine amylopectin structure, by either slab- or capillary-gel electrophoresis, into individual CL on a molar-based distribution. However, the detection response decreases with CL of oligoglucans above 13 (O'Shea et al., 1998). Chiou et al. (2005) have reported a normalization-independent plotting method to present the M distribution of amylopectin determined using capillary electrophoresis. This is reported to avoid arbitrary normalization of the non-linear detector responses to CL. The SEC-RI system has been used to study the weight-based distribution of amylopectin fine structure. However, it does not resolve the structure into individual CL, but determines the average-CL distribution. Hanashiro, Tagawa, Shibahara, Iwata, & Takeda (2002) labeled amylopectin with a fluorophore to the reducing termini and determined the weight- and molar-based distributions of A, B and C chains using the SEC-fluorescent detector-RI system. In the present study, we characterized the molecular structures of A and B chains using SEC-MALLS-RI system. We also demonstrated the capability of this method for characterizing the weight- and molar-based distributions of CL_w of the fine structure of amylopectin.

3.6. Complete method

Several starch characteristics can be determined relatively quickly with good starch recoveries using the method described in this paper. For example, one technical person could perform a molecular characterization of both amylose and amylopectin of 40 samples in 1-week time. The repeatability of this method is also quite good with an average measurement error (i.e., experimental uncertainty) of $<5\%$ for most of the molecular characterizations over samples ranging widely in amylose content. It should be noted that these error measurements do not include systemic error that might have resulted from the use of the dn/dc value obtained from the literature.

4. Conclusion

Amylose content is a key indicator of cooked rice texture, and amylopectin CL distribution explains much of the variation seen in rice starch GT. But these two aspects of starch do not explain all of the variation for these and other rice functional properties. It is possible that aspects of amylose structure might also explain some of the variation found in rice starch functionality. The method described above enables the measurement of starch characteristics that are known to impact rice functionality as well as others that are hypothesized to, including: weight- and molar-based distributions of DP, and M_w and M_n of amylose and amylopectin fine structure. With this method, large sample sets can be analyzed within a relatively short time frame with good repeatability, thus making it suitable for use in studies directed at understanding rice starch functionality and the genetics controlling these traits. This method should

Table 5
Alkali-spreading value (ASV) and molarity of subfractions of isoamylase-debranched amylopectin, obtained by summing the moles of the weight-average chain length (CL_w) subfractions indicated in parenthesis and expressing on a per gram of amylopectin basis, of eight rice cultivars

Molarity (μmol/g amp)	Total	B4[a] (CL_w:147–71)	B3 + B2[a] (CL_w:64–40)	B1[a] (CL_w:35–15)	A1[a] (CL_w:13–9)	(CL_w:25–15)	(CL_w: 12–11)	Ratio (CL_w:25–15)/(CL_w:12–11)	ASV
Dixie belle									
Molarity	277.1	4.0	25.8	176.7	70.7	146.3	36.0	4.08	4.0
SD	3.9	0.3	0.5	2.5	5.0	1.8	1.7	0.22	
CV (%)	1.42	6.47	2.00	1.43	7.14	1.25	4.76	5.46	
Kataktara									
Molarity	276.6	4.0	25.4	177.1	70.1	146.3	36.0	4.07	4.1
SD	2.1	0.4	0.8	1.5	2.6	1.2	1.5	0.17	
CV (%)	0.75	8.85	2.98	0.87	3.73	0.81	4.12	4.09	
L201									
Molarity	279.1	3.7	25.4	177.9	72.1	147.4	37.3	3.96	4.0
SD	4.5	0.2	0.6	1.4	5.5	1.2	2.5	0.28	
CV (%)	1.63	4.14	2.26	0.77	7.68	0.84	6.60	7.01	
Dellmont									
Molarity	283.9	3.4	24.9	177.4	78.1	146.9	40.3	3.65	4.7
SD	3.4	0.2	0.6	3.1	4.8	2.3	2.1	0.21	
CV (%)	1.20	4.85	2.55	1.73	6.13	1.60	5.11	5.84	
Bengal									
Molarity	281.8	4.0	25.9	166.1	85.9	135.3	45.0	3.01	6.0
SD	1.7	0.2	0.5	1.7	2.0	1.3	0.7	0.07	
CV (%)	0.60	4.23	1.85	0.99	2.34	0.95	1.58	2.44	
Rico1									
Molarity	286.3	3.8	25.6	167.9	89.0	137.1	46.4	2.95	6.0
SD	1.8	0.4	0.2	1.5	0.9	1.0	0.8	0.05	
CV (%)	0.62	10.70	0.83	0.92	0.99	0.76	1.65	1.56	
Hsiao Wu Tsu Tsi									
Molarity	280.3	3.6	23.9	170.8	82.1	141.5	43.3	3.28	4.0
SD	8.1	0.2	0.8	2.8	8.2	2.4	3.7	0.26	
CV (%)	2.89	5.98	3.29	1.62	9.99	1.70	8.53	7.82	
Daw Dam									
Molarity	289.1	3.2	24.2	165.4	96.3	135.5	50.8	2.67	7.0
SD	1.6	0.1	0.6	1.0	2.0	1.1	1.1	0.05	
CV (%)	0.54	4.03	2.32	0.62	2.03	0.82	2.14	2.01	

[a] The subfractions of B4, B3 + B2, B1, and A were divided at the inflection points of MALLS, Rl responses and molar-based distribution, respectively.

also be applicable for starch structural characterizations of diverse botanical sources.

Acknowledgements

We thank Janis Delgado and Naomi Gipson for excellent technical support. The advice on instrument setup, maintenance and data processing from Jeff Ahlgren and Michelle Chen of Wyatt Technology Corp. is greatly appreciated. Also, we thank Shannon Pinson for her assistance in growing and harvesting the rice samples. This research was partly funded by the US Rice Foundation.

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